

DATA EVALUATION RECORD
72-2 -- ACUTE LC₅₀ TEST WITH A FRESHWATER INVERTEBRATE
OCSPP 850.1020

1. **CHEMICAL**: Cypermethrin

PC Code No.: 109702

2. **TEST MATERIAL**: Cypermethrin

Purity: 95.2%
(49.8:50.2 cis:trans)

3. **CITATION**

Authors: Bradley MJ

Title: Cypermethrin- Acute Toxicity to Freshwater Amphipods
(*Hyalella azteca*) Under Flow-Through Conditions

Study Completion Date: October 17, 2013

Laboratory: Smithers Viscient
Wareham, Massachusetts

Sponsor: Pyrethroid Working Group
FMC Corporation
Ewing, New Jersey

Laboratory Report ID: 13656.6171

MRID No.: 49274301

DP Barcode: D417859

4. **REVIEWED BY**: John Marton, Ph.D., Environmental Scientist, CDM Smith

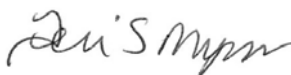
Signature:



Date: 04/22/15

APPROVED BY: Teri S. Myers, Ph.D., Environmental Scientist, CDM Smith

Signature:



Date: 05/04/15

5. **APPROVED BY**: {.....}, {Specialty}, OPP/EFED/ERB-{Section}

Signature:

Date:

6. **DISCLAIMER**: This document provides guidance for EPA and PMRA reviewers on how to complete a data evaluation record after reviewing a scientific study concerning the acute toxicity of a pesticide to freshwater invertebrates. It is not intended to prescribe conditions to any external party for conducting this study nor to establish absolute criteria regarding the assessment of whether the study is scientifically sound and whether the study satisfies any applicable data requirements. Reviewers are expected to review and to determine for each study, on a case-by-case basis, whether it is scientifically sound and provides sufficient information to

satisfy applicable data requirements. Studies that fail to meet any of the conditions may be accepted, if appropriate; similarly, studies that meet all of the conditions may be rejected, if appropriate. In sum, the reviewer is to take into account the totality of factors related to the test methodology and results in determining the acceptability of the study.

7. STUDY PARAMETERS

| | |
|------------------------------------------|------------------------|
| Scientific Name of Test Organism: | <i>Hyalella azteca</i> |
| Age of Test Organism: | 7 days old |
| Definitive Test Duration: | 96 hours |
| Study Method: | Flow-through |
| Type of Concentrations: | Mean measured |

8. CONCLUSIONS:

Results Synopsis

96-hour LC₅₀: 0.560 ng ai/L

Probit Slope: 4.12

95% C.I.: 0.453-0.711 ng ai/L

95% C.I.: 2.73-5.51

9. ADEQUACY OF THE STUDY

A. Classification: This study [is/is not scientifically sound] and is classified as [acceptable/supplemental (quantitative)/supplemental (qualitative)/invalid].

B. Rationale:

C. Repairability:

10. Guideline Deviations: This study was conducted following a protocol that generally meets the testing requirements of the U.S. EPA's Ecological Effects Test Guideline (Draft) OCSPP 850.1020 Gammarid Acute Toxicity Test; and the U.S. EPA's Ecological Effects Test Guideline (Draft) OCSPP 850.1000 Special Considerations for Conducting Aquatic Laboratory Studies. The following deviations from OCSPP 850.1020 were noted:

1. *Hyalella azteca* is not one of the preferred non-daphnid test organisms.
2. The instar of the test organisms was not specified.
3. Test organisms were fed during the definitive test.
4. Recommended temperature during the definitive test (22-24°C) exceeded the recommended temperature of 18±1°C. However, these recommendations are based on the genus *Gammarus*.
5. Biomass loading rate was not specified. However, only 10 amphipods were in each test vessel (1.8 L fill volume).

These deviations do/do not impact the acceptability of the study.

11. **SUBMISSION PURPOSE**: This study was submitted to provide data on the effects of cypermethrin to *Hyaella azteca* following acute exposure for the purpose of chemical re-registration.

12. **MATERIALS AND METHODS**

A. Test Organisms

| Guideline Criteria | Reported Information |
|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------------------|
| <u>Species</u> Preferred species is <i>Daphnia magna</i> | <i>Hyaella azteca</i> |
| All organisms are approximately the same size and weight? | Yes |
| <u>Life Stage</u> Daphnids: 1 st instar (<24 h). Amphipods, stoneflies, and mayflies: 2 nd instar. Midges: 2 nd & 3 th instar. | 7 days old |
| Supplier | Laboratory cultures |
| All organisms from the same source? | Yes |

B. Source/Acclimation

| Guideline Criteria | Reported Information |
|-----------------------------------------------------------|----------------------|
| <u>Acclimation Period</u> Minimum 7 days | 8 days |
| Wild caught organisms were quarantined for 7 days? | N/A |
| Were there signs of disease or injury? | None reported |

| Guideline Criteria | Reported Information |
|-----------------------------------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| If treated for disease, was there no sign of the disease remaining during the 48 hours prior to testing? | N/A |
| <u>Feeding</u> No feeding during the study. | During the holding period, the amphipods were fed a combination of yeast, cereal leaves, and flaked food suspension (YCT), as well as a unicellular green algae, <i>Ankistrodesmus falcatus</i> , and finely ground flaked fish food suspension (3 drops at a rate of 100 mg/mL) on the first day of holding. During the definitive exposure, each replicate test vessel received 1.0 mL of YCT daily. |
| <u>Pretest Mortality</u> No more than 3% mortality 48 hours prior to testing. | None reported |

C. Test System:

| Guideline Criteria | Reported Information |
|------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <u>Source of dilution water</u> Soft reconstituted water or water from a natural source, not dechlorinated tap water. | Laboratory well water. <i>H. Azteca</i> are cultured in water from the same source as the dilution water used in the study and have successfully survived and reproduced over multiple generations. |
| Does water support test animals without observable signs of stress? | Yes |
| <u>Water Temperature</u> Daphnia: 20°C Amphipods and mayflies: 17°C Midges and mayflies: 22°C Stoneflies: 12°C | 22-24°C |
| <u>pH</u> Prefer 7.2 to 7.6. | 7.0-7.3 |
| <u>Dissolved Oxygen</u> Static: $\geq 60\%$ during 1 st 48 h and $\geq 40\%$ during 2 nd 48 h, flow-through: $\geq 60\%$. | 6.4-8.3 mg/L ($\geq 75\%$ of saturation) The lowest DO readings (6.4 mg/L) were measured in the nominal 1.2 and 2.4 ng ai/L treatment levels at 72 and 48 hours, respectively. |
| <u>Total Hardness</u> Prefer 40 to 48 mg/L as CaCO ₃ . | 40-44 mg/L as CaCO ₃ Alkalinity: 18-22 mg/L as CaCO ₃ Conductivity: 240 μ S/cm |

| Guideline Criteria | Reported Information |
|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <p><u>Test Aquaria</u></p> <p>1. <u>Material</u>: Glass or stainless steel.</p> <p>2. <u>Size</u>: 250 ml (daphnids and midges) or 3.9 L (1 gal).</p> <p>3. <u>Fill volume</u>: 200 ml (daphnids and midges) or 2-3 L.</p> | <p>1. Glass beaker</p> <p>2. 2 L</p> <p>3. 1.8 L (depth of 14.5 cm)</p> <p>Each test vessel had a slot cut below the top edge of the beaker which was covered with 40-mesh NITEX® screen, adhered with silicone, for drainage. Each test vessel also contained a 3 cm² piece of 250-µm stainless steel mesh as a substrate.</p> |
| <p><u>Type of Dilution System</u></p> <p>Must provide reproducible supply of toxicant.</p> | <p>Intermittent-flow proportional diluter.</p> |
| <p><u>Flow Rate</u></p> <p>Consistent flow rate of 5-10 vol/24 hours, meter systems calibrated before study and checked twice daily during test period.</p> | <p>~10 vol/24 hours (90% replacement time of approximately 5 hours)</p> <p>System was calibrated prior to use and was confirmed at test termination. Diluter system function was monitored daily and a visual check of the system's operation was performed twice daily.</p> |
| <p><u>Biomass Loading Rate</u></p> <p>Static: # 0.8 g/L at # 17°C, # 0.5 g/L at > 17°C; flow-through: # 1 g/L/day.</p> | <p>Not reported</p> |
| <p><u>Photoperiod</u></p> <p>16 hours light, 8 hours dark.</p> | <p>16L:8D with 15-30-minute transition periods of low-light intensity.</p> <p>Light intensity ranged from 220-290 lux.</p> |

| Guideline Criteria | Reported Information |
|-------------------------------------------------------------------------------------------------------|----------------------|
| <u>Solvents</u> Not to exceed 0.5 ml/L for static tests or 0.1 ml/L for flow-through tests. | Acetone (0.050 mL/L) |

D. Test Design:

| Guideline Criteria | Reported Information |
|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <u>Range Finding Test</u> If $LC_{50} > 100$ mg/L, then no definitive test is required. | A 96-hour range-finding study was conducted using nominal concentrations of 0 (negative and solvent controls), 0.63, 1.3, 2.5, 5.0, and 10 ng ai/L with 20 amphipods per level (2 reps w/10 each). After 96 hours of exposure mortality was 0% in both controls and 0, 75, 95, 100, and 100% in the 0.63, 1.3, 2.5, 5.0, and 10 ng ai/L groups, respectively. All surviving amphipods at the 1.3 and 2.5 ng ai/L treatment levels were lethargic. |
| <u>Nominal Concentrations of Definitive Test</u> Control & 5 treatment levels; a geometric series with each concentration being at least 60% of the next higher one. | 0.30, 0.60, 1.2, 2.4, and 4.8 ng ai/L |
| <u>Number of Test Organisms</u> Minimum 20/level, may be divided among containers. | 20/level, with 10 amphipods in each of two replicates |
| Test organisms randomly or impartially assigned to test vessels? | Yes |

| Guideline Criteria | Reported Information |
|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <u>Water Parameter Measurements</u> 1. <u>Temperature</u> Measured continuously or, if water baths are used, every 6 h, may not vary > 1°C. 2. <u>DO and pH</u> Measured at beginning of test and ever 48 h in the high, medium, and low doses and in the control. | 1. Measured in replicate A of all treatment levels at test initiation and in alternating replicates daily thereafter. Temperature was also continuously monitored in replicate B of the nominal 4.8 ng ai/L treatment level. 2. Dissolved oxygen and pH were measured in replicate A of all treatment levels at test initiation and in alternating replicates daily thereafter. |
| <u>Chemical Analysis</u> Needed if solutions were aerated, if chemical was volatile, insoluble, or known to absorb, if precipitate formed, if containers were not steel or glass, or if flow-through system was used | Samples were collected from each control and treatment level at 0 and 96 hours. |

13. REPORTED RESULTS:

| Guideline Criteria | Reported Information |
|-------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Quality assurance and GLP compliance statements were included in the report? | Yes. Signed and dated No Data Confidentiality, GLP, and Quality Assurance statements were provided. This study was conducted in compliance with all pertinent U.S. EPA Good Laboratory Practice Regulations (40 CFR, Part 160) with the following exceptions: routine water and food contaminant screening analyses were conducted using standard U.S. EPA procedures by GeoLabs, Inc., Braintree, Massachusetts. |
| <u>Control Mortality</u> Static: ≤10% Flow-through: ≤5% | Negative Control: 0% Solvent Control: 0% |

DP Barcode: D417859

MRID No.: 49274301

| | |
|-------------------------------------|------------------------------------------------------------------------------------------------------------------------------------|
| Percent Recovery of Chemical | 52-85% of nominal based on mean-measured concentrations. QC spikes yielded recoveries ranging from 94.2 to 103% of nominal. |
| Raw data included? | Yes |

Mortality

| Concentration (ng ai/L) | | Number of Organisms | Cumulative Number Dead | | | |
|-------------------------|---------------|---------------------|------------------------|----|----|----|
| Nominal | Mean Measured | | Hour of Study | | | |
| | | | 24 | 48 | 72 | 96 |
| Control | <LOQ | 20 | 0 | 0 | 0 | 0 |
| Solvent Control | <LOQ | 20 | 0 | 0 | 0 | 0 |
| 0.30 | 0.21 | 20 | 0 | 1 | 1 | 1 |
| 0.60 | 0.31 | 20 | 0 | 0 | 2 | 2 |
| 1.2 | 0.68 | 20 | 2 | 6 | 12 | 14 |
| 2.4 | 1.6 | 20 | 11 | 17 | 18 | 19 |
| 4.8 | 4.1 | 20 | 15 | 20 | 20 | 20 |

Other Significant Results: No sub-lethal effects were observed in the controls or the mean-measured 0.21 and 0.31 ng ai/L treatment groups throughout the 96-hour exposure period. Several surviving amphipods in the mean-measured 0.68 ng ai/L group were lethargic or immobilized at all observation periods, whereas all surviving amphipods were affected at the 1.6 ng ai/L level from 48-96 hours. Of the surviving amphipods at the mean-measured 4.1 ng ai/L treatment level at 24 hours, several were lethargic and one was immobilized.

B. Statistical Results

Method: The 96-hour LC₅₀ and associated 95% C.I. were estimated using the Trimmed Spearman-Kärber method via CETIS statistical software version 1.8. Mean-measured concentrations were used in the analysis.

96-hour LC₅₀: 0.56 ng ai/L
Probit Slope: N/A

95% C.I.: 0.45-0.69 ng ai/L
95% C.I.: N/A

14. VERIFICATION OF STATISTICAL RESULTS

| Parameter | Result |
|-----------------------------------------------------|-----------------------------|
| Trimmed Spearman-Kärber LC ₅₀ (95% C.I.) | 0.558 (0.451-0.690) ng ai/L |
| Probit LC ₅₀ (95% C.I.) | 0.560 (0.453-0.711) ng ai/L |
| Probit Slope (95% C.I.) | 4.12 (2.73-5.51) |

The reviewer analyzed mortality data using the probit analysis via CETIS statistical software version 1.8.7.12 with database backend settings implemented by EFED on 3/25/14. Treatment data were compared to the negative control only. Results from the Trimmed Spearman-Kärber method were also reported. Toxicity values were based on the reported mean-measured concentrations.

15. REVIEWER'S COMMENTS:

The reviewer's results were based on the probit analysis whereas the study author reported results from the Trimmed Spearman-Kärber method. Therefore, the reviewer's results are reported in the Conclusions section of this DER.

An initial definitive toxicity test was conducted from May 9 to 13, 2013 with nominal concentrations of 0.30, 0.60, 1.2, 2.4, and 4.8 ng ai/L. However, this exposure was terminated due to contamination of the solvent control and repeated as the definitive exposure period.

The TOC concentration for the dilution water source was 0.31 mg/L for August 2013.

Results from the periodic screening analysis of the dilution water were not provided. However, the study author reported that no pesticides, PCBs, or toxic metals were detected at concentrations that are considered toxic in any of the water samples analyzed in agreement with ASTM (2002) standard practices.

The in-life portion of the definitive toxicity test was conducted from August 26 to 30, 2013.

This study [is/is not scientifically sound] and is classified as [acceptable/supplemental (quantitative)/supplemental (qualitative)/invalid].

16. REFERENCES:

Dix ME. 2013. Method Validation for Eight Pyrethroids in Freshwater by Gas Chromatography using Mass Selective Detection with Negative Ionization. Smithers Viscient, Wareham, Massachusetts. Study No. 13656.6174.

Ives M. 2013. Comprehensive Environmental Toxicity Information SystemTM, User's Guide. Tidepool Scientific Software, McKinleyville, California.

Mount DI, Brungs WA. 1967. A simplified dosing apparatus for fish toxicity studies. *Water Research* 1:20-29.

Sprague JB. 1969. Measurement of pollutant toxicity to fish. 1. Bioassay methods for acute toxicity. *Water Research* 3:793-821.